MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

Contract #N01-NS-8-2301

3rd Progress Report
April 1, 1999 to June 30, 1999
Neural Prosthesis Program

Prepared for
The National Institutes of Health
National Institute of Neurological Disorders and Stroke
Bethesda, Maryland

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I. Introduction

During this quarter both colon tracing and microstimulation experiments continued. These studies used tracers to determine the location and distribution of neurons and interneurons in the lumbosacral spinal cord which provide input to the colon smooth muscle and the striated muscle of the external anal sphincter (EAS). These neurons are then electrically stimulated using fine tipped microelectrodes to determine the location of sites which modulate colon and EAS activity. During this quarter in addition to examining the intraluminal pressure changes in the distal and proximal colon, recordings were also made of the pressure changes generated at the level of the anal sphincter. A variety of sites in the sacral spinal cord can modulate colon and sphincter contraction. The S_2 and S_3 segments provide the major excitatory input to the colon smooth muscle while S_1 and S_2 provide the excitatory input to the striated muscle of the external anal sphincter. An interesting result of our most recent experiment, in which the colon and sphincter activity were recorded simultaneously, indicates that microstimulation of the specific site in the sacral cord can produce excitation of the EAS while other sites produce inhibition. These studies are described in detail below as well as some thought as to possible mechanisms for these various responses.

II. Modulation of Colon Intraluminal and Sphincter Pressure by Sacral Spinal Cord Microstimulation

The goal of these studies is to determine sites in the spinal cord which modulate colon and sphincter activity when activated by electrical pulses delivered via fine tipped microelectrodes.

The methods used have been described in detail in previous progress reports and are in

part diagrammatically outlined in Figure 1. During this quarter several modifications and additions have been made to our methodology. As depicted in Figure 1 a third saline filled balloon has been added to the experimental design in order to record changes in sphincter pressure. The balloon is inserted in the anal canal via an external approach as shown in Figure 1. About 75% of the length of the balloon is within the anal canal while the remaining 25% is left external. This allows recording pressure changes generated by the striated muscles of external anal sphincter (EAS) to be recorded. It should be pointed out that the pressures recorded by this balloon may not be exclusively from the striated muscles of EAS since this muscle is in close proximity to, and interspersed with the smooth muscle of the internal anal sphincter (IAS) and the lower segment of the anal canal. Therefore the resultant pressure is an algebraic sum of both smooth and striated muscle acting on the balloon. Throughout this progress report we will therefore use the general term "sphincter pressure"rather than more specific terms such as external or internal anal sphincter pressure. It is obvious, in many instances, when examining pressure recordings for this most distal positioned balloon, that the rapid increase in pressure when a stimulus is applied to the spinal cord or ventral roots and the rapid relaxation that occurs when the stimulus is terminated is characteristic of striated muscle rather than smooth muscle contractions. Smooth muscle contractions are usually somewhat slow in onset and require a longer time for complete relaxation.

The issue of the type of anesthesia and the inhibition of G.I. motility during surgical manipulation is examined in each experiment. The amount of stretching, manipulation and surgery on the colon is however kept to a minimum. The sympathetic input to the colon is reduced by sectioning the sympathetic nerves (hypogastric and lumbar colonic) to the colon.

This was found necessary in order to produce an adequately excitatory contraction from ventral root or spinal cord stimulation. Likewise the type and level of anesthetic or anesthetic combinations can change the excitability of G.I. smooth muscle. In most of our experiments we have used pentobarbital although α-chlorolose has been used in some of our earlier experiments.

The goal of blocking sympathetic inhibition and choosing the correct anesthetic is to allow the colon to respond to excitatory input while having minimal spontaneous activity that would interfere with measuring excitatory responses.

Prior to stimulating the spinal cord with microelectrodes, each sacral ventral root is stimulated using a hook electrode at various intensities of stimulation to determine the spinal cord segments which produce the largest amplitude colon or sphincter contractions. This segment will then be used for probing with stimulating microelectrodes. In recent experiments, we have identified, isolated, and stimulated the right S_1, S_2, and S_3 ventral roots. The left ventral roots were left untouched so as to prevent even the slightest damage that may occur from isolation and stimulation of the roots. The left side of the cord was then used for spinal cord stimulation. The ventral root outflow should, therefore, be in the best possible condition for spinal cord mapping. To identify the S_2 segment precisely on the left side, the S_2 dorsal root is located and followed back to the dorsal root entry zone (DREZ), the site where the afferent fibers attach to the spinal cord. Figure 2 shows data from a single animal in which the sacral (S_1, S_2, and S_3) ventral roots were stimulated at increasing intensities. Although some variability exists, Figure 2 represents a rather typical animal experiment where the ventral roots of each sacral segment (S_1, S_2, and S_3) were stimulated, while recording pressure changes from three balloons placed in the distal colon, proximal colon, and sphincters. Since the proximal colon had little or

no response, only sphincter (s) and distal colon (d) responses are shown in the figure. Stimulation of the S_1 ventral root produces the largest sphincter responses with very little or no colon responses. The S_2 segment, however, produces the largest distal colon response with a significant sphincter response. The sphincter response is, however, smaller than that seen in the S_1 segment. The S_3 segment produces a somewhat small distal colon response and no sphincter response. These results are fairly typical for most animals, although S_3 will frequently produce a response in distal colon nearly as large as S_2 ventral root stimulation.

Once it has been established which segment produces the largest excitability outflow to the colon, using ventral root stimulation, that segment of the spinal cord, usually S_2, is examined using fine tipped (400μm^2 exposed surface) activated iridium electrodes. Figure 3 shows a typical response of colon and sphincter to microstimulation of the S_2 segment near or within the sacral parasympathetic nucleus (SPN). The proximal colon, which receives a small input from the sacral spinal cord, usually has a small response. The distal colon typically produces a large (>20cm H_2O) response while the sphincter response is quite variable from S_2 stimulation, producing both excitation and inhibition. The colon and sphincter responses produced by spinal cord microstimulation are quite dependent on stimulus parameters, especially changes in frequency and intensity of stimulation. Figures 4 and 5 show the effects of frequency changes on both colon and sphincter activity. The best frequency for maximizing the colon pressure responses is between 10 and 20 Hz. This is a fairly consistent finding with both spinal cord and ventral root stimulation. The sphincter response in Figures 4 and 5 also show marked differences in responses with frequency changes. The sphincter response is maximum at 5 or 10 Hz and decreases with increasing frequency of cord stimulation and producing a large relaxation at the

colon outlet. A major component of the sphincter activity shown in Figure 4 and plotted in graphic form in Figure 5 is probably smooth muscle activity intermixed with a small amount of striated muscle. The rapid pressure change with stimulus onset is probably striated muscle while the component with the slow onset is probably smooth muscle. The smooth muscle component can be determined best by the slow decline in pressure following termination of the stimulus. The inhibition of sphincter contractions can be interpreted several ways. One is simple muscle fatigue that is seen with increasing frequency of stimulation. A second interpretation could be that the higher frequencies of stimulation activate local inhibitory neurons which produce an active decrease in sphincter activity. A third possibility is that the increase in colon pressure triggers an inhibitory reflex which decreases sphincter activity. Mapping experiments in which the spinal cord was stimulated at various depths from the cord surface produce different pattern responses depending on the depth and mediolateral position of the stimulating electrode (Figures 6 - 10). Figures 6 - 10 graph colon and sphincter data from five microelectrode tracts in the S_2 spinal cord. Tract #1 (Figure 6) is at the dorsal root entry zone (DREZ) with Tracts #2 & #3, 300 and 600 μ medial to Tract #1 respectively. Tracts #4 & #5 are 300 and 600 μ lateral to Tract #1. Data from these series of tracts (Figures 6 - 10) illustrate a number of important points:

1. Enhanced colon pressure can best be seen at a depth of 1.0 - 1.6 mm from the spinal cord surface and extends 300 to 600 μ medial and lateral from the DREZ. This corresponds to sites near or in the cell bodies and axons of the sacral parasympathetic nucleus. This is best illustrated in the figures (6-10) where peak pressure response (middle panel each figure) is plotted as a function of depth from the spinal cord surface. Area under the pressure curve vs depth (top panel each figure (6-10)) is also a good indication of colon or sphincter activity.

2. Sphincter activity is strongly inhibited at sites where large colon pressures are

generated (Figure 6, 7, & 8). This is similar to what occurs in a normal defecation reflex (i.e., the colon contracts and the sphincter which controls the outlet of the colon relaxes)

3. Sphincter activity and at times colon is high near the dorsal surface of the spinal cord and also often deep near the ventral surface of the cord. The activity near the dorsal surface probably results from stimulation of afferent pathways in the dorsal horn and dorsal columns generating reflex activity at several levels of the spinal cord. The activity generated deep in the ventral horn near the ventral surface, may in part be current spread along the adjacent ventral roots since sphincter and colon activity are often accompanied by somatic motor activity of the hindlimb.

4. The sites which produce large colon contractions and sphincter inhibition correspond to sites labeled by pseudorabies virus injected into the wall of the colon.

5. The relaxation of sphincter does not seem to be simple fatigue since stimulation of superficial sites near the cord surface produce sphincter contractions with no significant fatigue, while sites deeper in the spinal cord produce relaxation. Whether these deeper sites produce direct inhibition of sphincter or is merely a reflex generated by a large colon contraction will be determined in future studies.

Many of these studies will continue into the next quarter.

Figure 1: Schematic diagram showing methods for recording changes in anal sphincter and intraluminal colon pressure. Two saline filled balloons are inserted in the colon via a small incision near the junction of the ileum with the proximal colon while a third balloon is positioned via an external approach in the anal canal with a portion of the balloon remaining external. This third balloon, added to our most recent experiments, records pressure produced by the striated muscle of the external anal sphincter (EAS) while the other two balloons within the colon record intraluminal pressure changes in the proximal and distal colon. The balloon catheters are each connected to a transducer, and the signals produced are amplified and recorded on paper (chart recorder) and on tape. The signals are also digitized and stored on the computer disk drive. The computer provides various displays during the experiment, and is also used for analysis following completion of the experiment.

Figure 2: Graphs of distal colon (d) and sphincter (s) pressure changes to stimulation of the S_1 (top), S_2 (middle), and S_3 (bottom) ventral roots (VRT) at increasing intensities of stimulation. Responses include peak pressure (Peak) area under the pressure curve (Area), and duration of pressure change (Duration) for both distal colon (d) and sphincter (s). Stimulus parameters using a hook electrode on ventral roots are: 15 Hz, 0.05 msec pulse duration, 0.1 to 20 volts 30 seconds on and 120 seconds off. Notice that S1 ventral root stimulation produces primarily a large sphincter response and only a small distal colon response, while S2 ventral root produces a large colon response and significant sphincter response.

Figure 3: Computer plots generated from digitized data showing change in colon and sphincter pressure to microstimulation of the S2 spinal cord. Pressure is recorded from three balloons from the proximal (top) and distal (middle) colon and from the region of the external anal sphincter (bottom) (as shown in Figure 1). The tip of the microelectrode is 1.2 mm from the spinal cord surface at the dorsal root entry zone (DREZ). Notice the response in distal colon is quite large while that of proximal colon is small. The sphincter response is likely a response of the smooth muscle of the internal anal sphincter (IAS) and the striated muscle of the external anal sphincter (EAS). Stimulus parameters are shown in figure.

Figure 4: Computer plots generated from digitized data showing changes in colon and sphincter pressure to microstimulation of the S2 spinal cord at various frequencies of stimulation from 5 to 40 Hz. Plots are similar to Figure 2. The electrode tip is at the level of the S2 spinal cord near the sacral parasympathetic nucleus (SPN), 1.2 mm from the cord surface. Pressure changes are recorded from three levels of the colon as shown in Figure 1. Notice that the peak response for distal colon is at 15 to 20 Hz, while sphincter activity is maximum in this particular experiment at 10 Hz and is inhibited or fatigues quickly at the higher frequencies of 20 to 40 Hz. Some proximal colon activity appears at 40 Hz although this is quite variable. Stimulus parameters are shown in figure.

Figure 5: Graphs of pressure changes at three sites in the large intestine (sphincter, distal colon, and proximal colon) to focal microstimulation of the S2 spinal cord at increasing frequencies of stimulation. The three graphs plot: area under the pressure curves (top panel), peak pressures (middle panel) and duration of pressure changes (bottom panel) as a function of frequency from 5 to 40 Hz. The electrode tip is 1.2 mm from the spinal cord surface, near the sacral parasympathetic nucleus. Stimulus parameters are: 0.2 msec pulse duration, 100 μA, 5 - 40 Hz, 30 seconds stimulation on and 120 seconds off. Notice little or no response of proximal colon at all frequencies but a large response of distal colon especially at 10 to 20 Hz. The sphincter seems to respond best to low frequency but it should be remembered that the sphincters are often inhibited by large distal colon contractions (defecation reflex). Therefore a variety of interactions may be occurring here (see text).

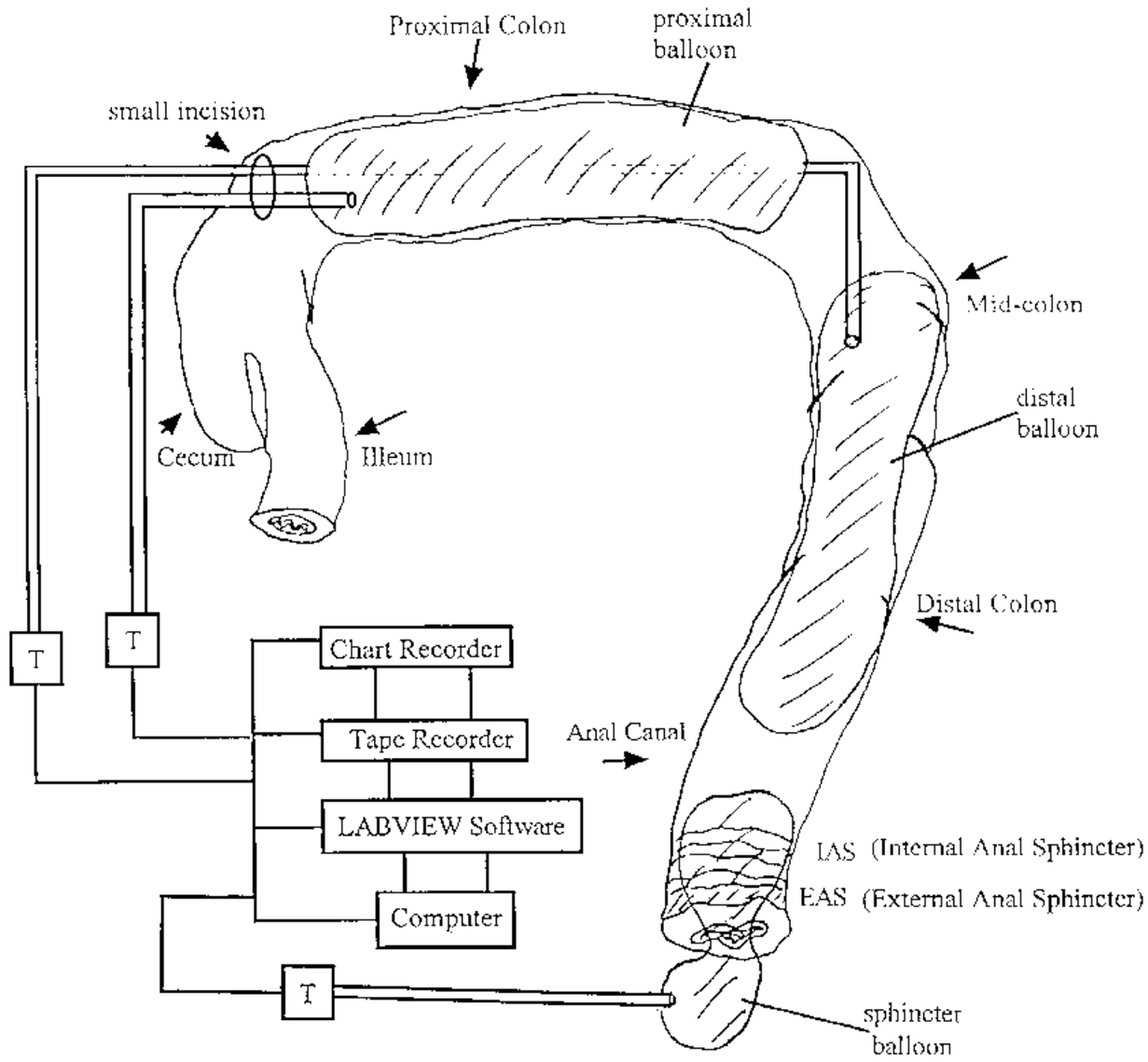
Figure 6: Graphs showing changes in intraluminal pressure from three sites along the large intestines (distal colon, proximal colon, sphincter) to focal microstimulation of the S2 spinal cord at different depths. Three types of graphs are shown for each tract. Area under the pressure curve (top panel), peak pressure (middle panel), duration of pressure change (bottom panel). Figures 6 - 10 are from five different tracts in the same rostrocaudal plane from the same animal. Tract #1 is at the dorsal root entry zone. Tract #2 (Figure 7) is 300 μ medial to Tract #1 and Tract #3 (Figure 8) is 300 μ medial to Tract #2. Tract #5 (Figure 9) is 300 μ lateral to Tract #1, Tract #6 (Figure 10) is 300 μ lateral to Tract #5. Stimulus parameters are 0.2 msec duration, 15 Hz, 100 μA. Notice that the proximal colon has a small amplitude response compared to distal colon. Large responses are often seen at very superficial sites (0.8 mm or less) and very deep sites (2.5 mm or greater). The best responses for colon are usually seen at 1.2 - 1.8 mm from surface.

Figure 7: Same as Figure 6 except Tract #2 is 300 μ medial to Tract #1.

Figure 8: Same as Figure 6 except Tract #3 is 600 μ medial to Tract #1.

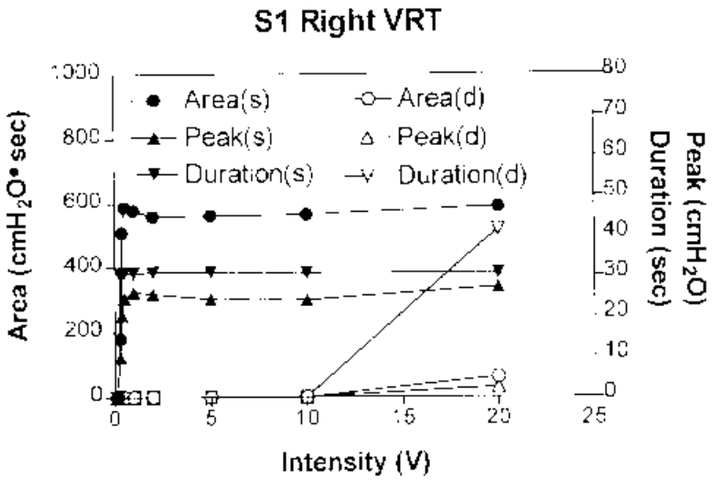
Figure 9: Same as Figure 6 except Tract #4 is 300 μ lateral to Tract #1.

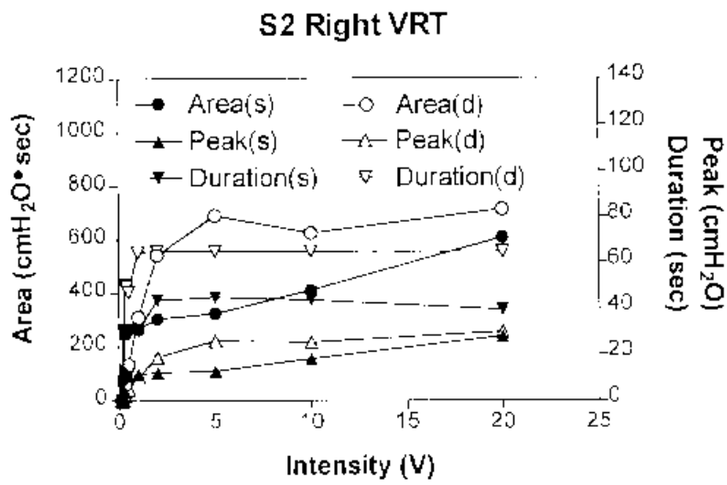
Figure 10: Same as Figure 6 except Tract #5 is 600 μ lateral to Tract #1.



T = Transducer

Figure 1


S1 Right VRT
Area(s)
Area(d)
Peak(s)
Peak(d)
Duration(s)
Duration(d)
Area (cmH2O*sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)


S2 Right VRT
Area(s)
Area(d)
Peak(s)
Peak(d)
Duration(s)
Duration(d)
Area (cmH2O*sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)

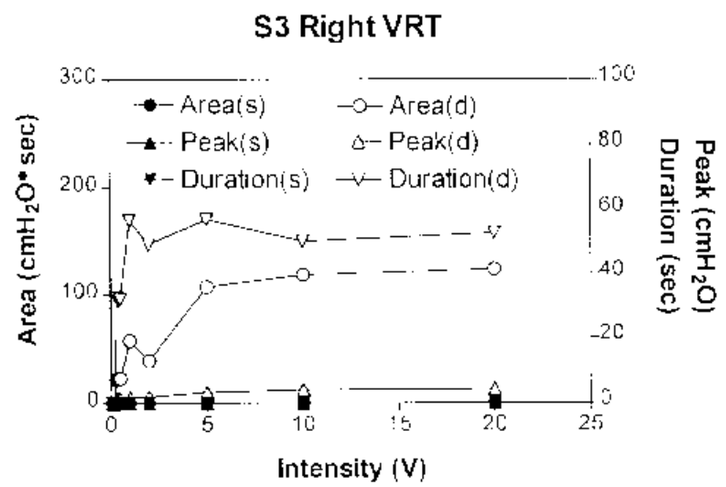
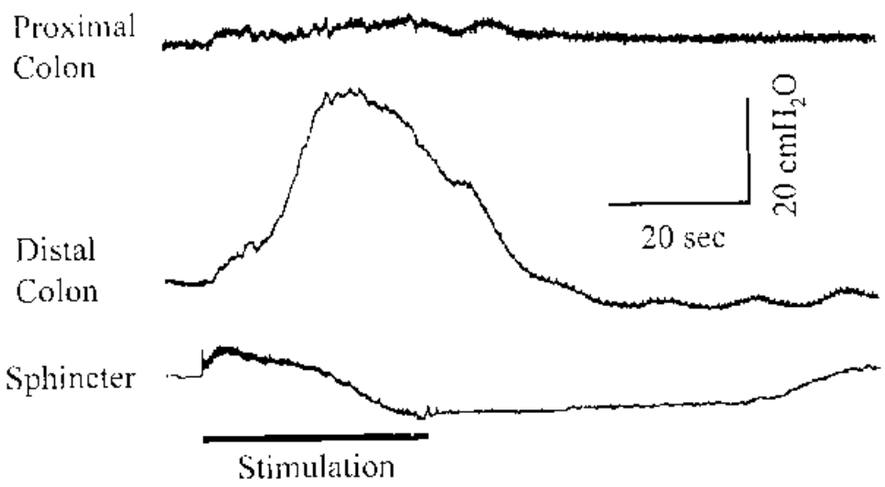
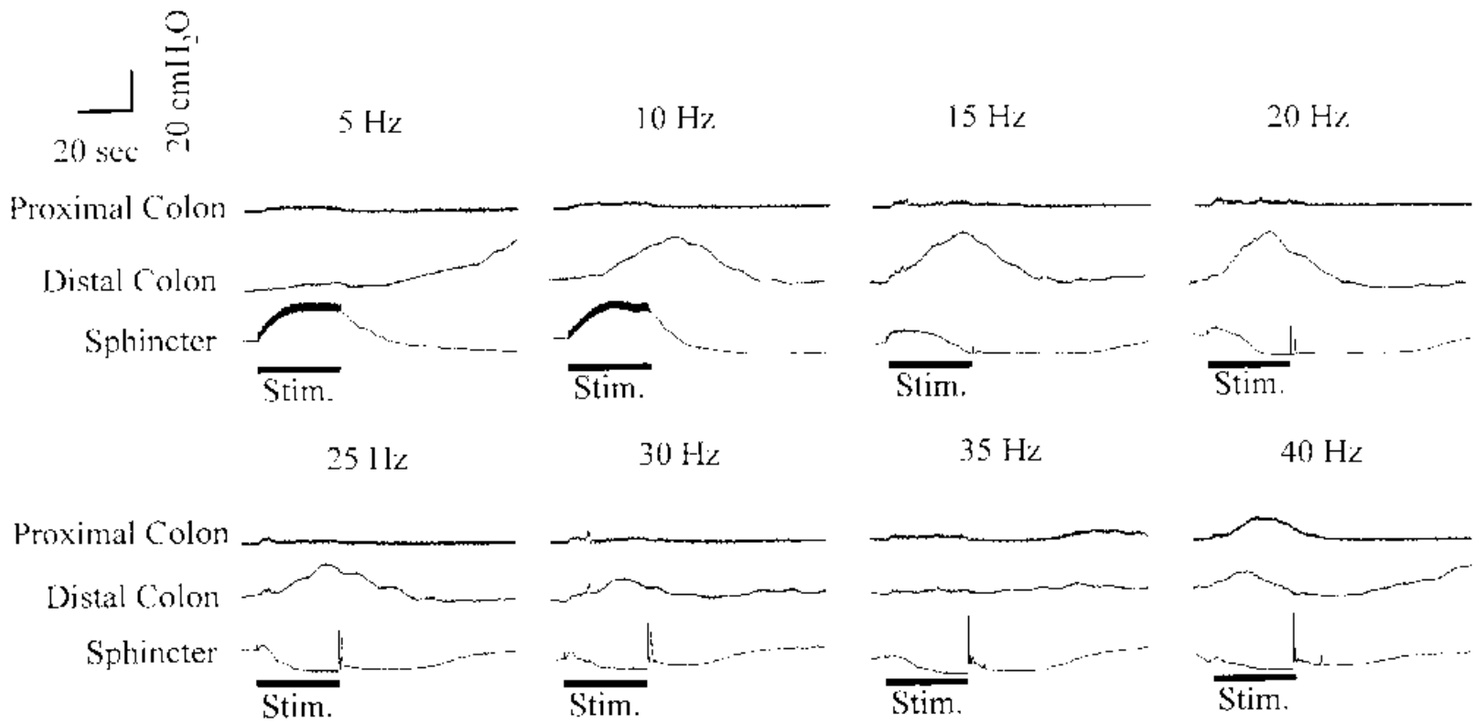

S3 Right VRT
Area(s)
Area(d)
Peak(s)
Peak(d)
Duration(s)
Duration(d)
Area (cmH2O*sec)
Peak (cmH2O)
Duration (sec)
Intensity (V)

Figure 3



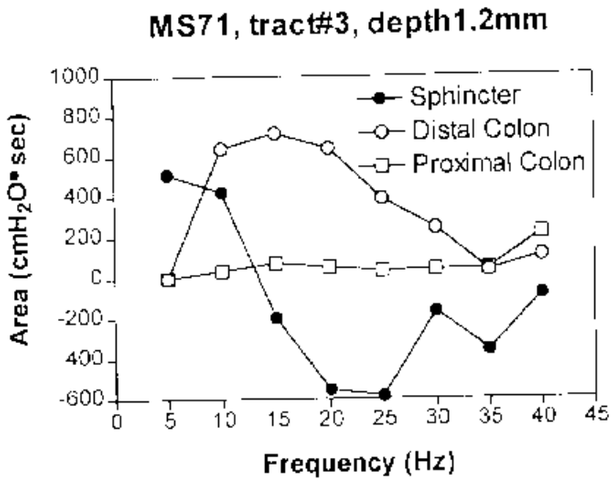
Stimulation: 100 μA intensity, 0.2 ms pulse width and 15 Hz frequency.
Data from MS#71, No.147, Tract#3 at depth of 1.2 mm.

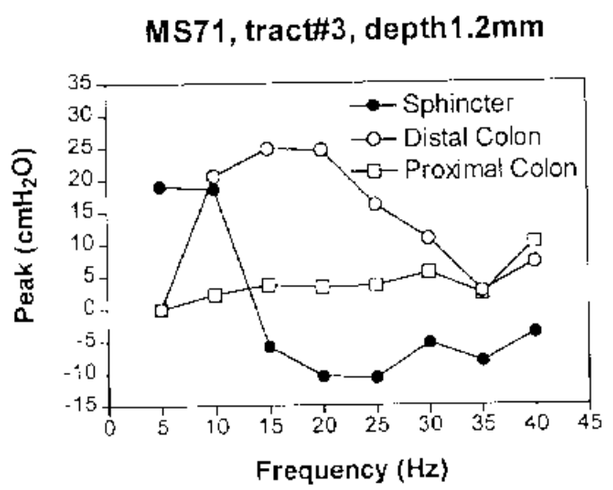
Figure 3



Stimulation: 0.2 ms pulse width, 100 μA intensity and 30 sec stimulation duration.
Data from MS#71. No.148.150.152,154-156.158 and 159. S2, Tract#3 at 1.2 mm depth.

Figure 4


MS71, tract#3, depth1.2mm
Sphincter
Distal Colon
Proximal Colon
Area (cmH2O*sec)
1000
800
600
400
200
0
-200
400
-600
0
5
10
15
20
25
30
35
40
45
Frequency (Hz)


MS71, tract#3, depth1.2mm
Sphincter
Distal Colon
Proximal Colon
Peak (cmH2O)
35
30
25
20
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
35
40
45
Frequency (Hz)

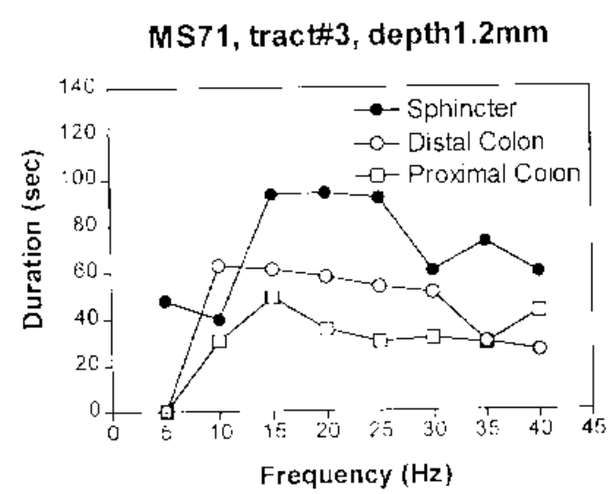
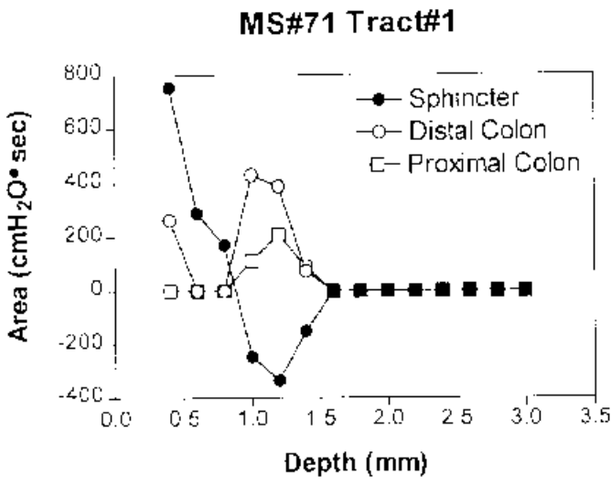
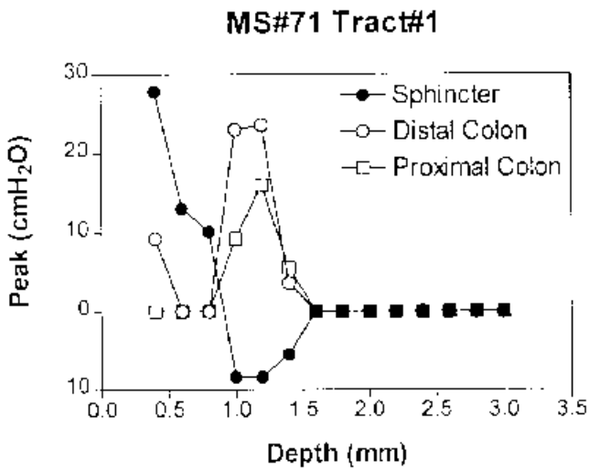

MS71, tract#3, depth1.2mm
Sphincter
Distal Colon
Proximal Colon
Duration (sec)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Frequency (Hz)

Figure 5


MS#71 Tract#1
Area (cmH2O• sec)
800
600
400
200
0
-200
-400
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon


MS#71 Tract#1
Peak (cmH2O)
30
20
10
0
10
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

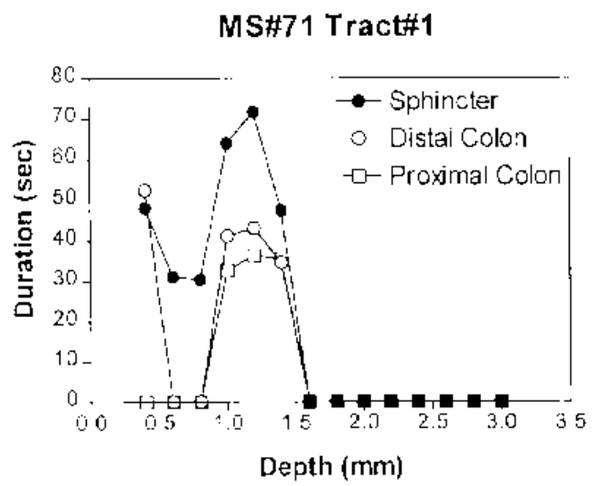
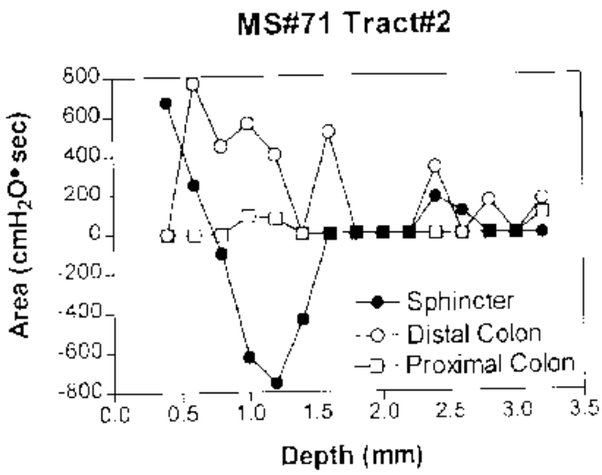
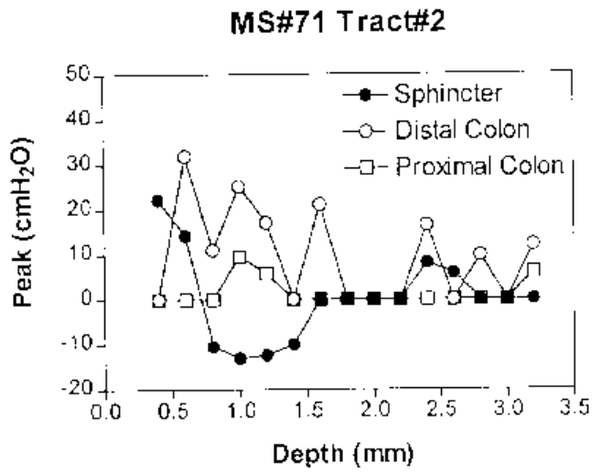

MS#71 Tract#1
Duration (sec)
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

Figure 6


MS#71 Tract#2
Area (cmH2O•sec)
600
600
400
200
0
-200
-400
-600
-800
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon


MS#71 Tract#2
Peak (cmH2O)
50
40
30
20
10
0
-10
-20
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

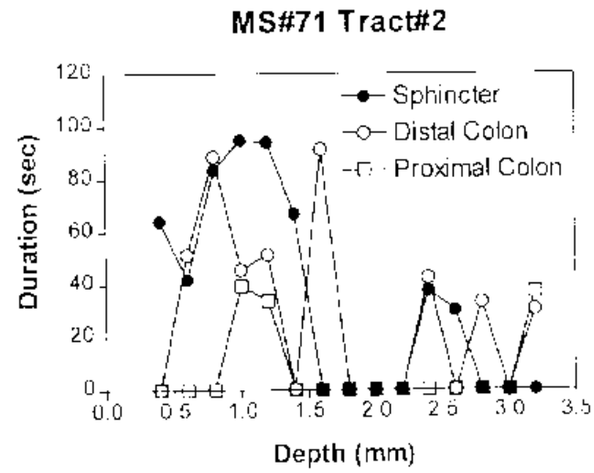
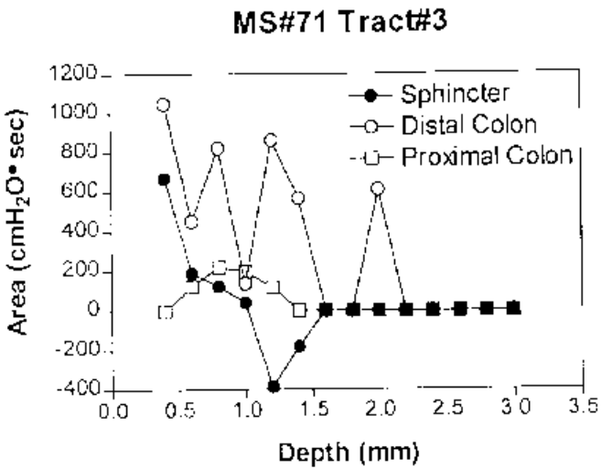
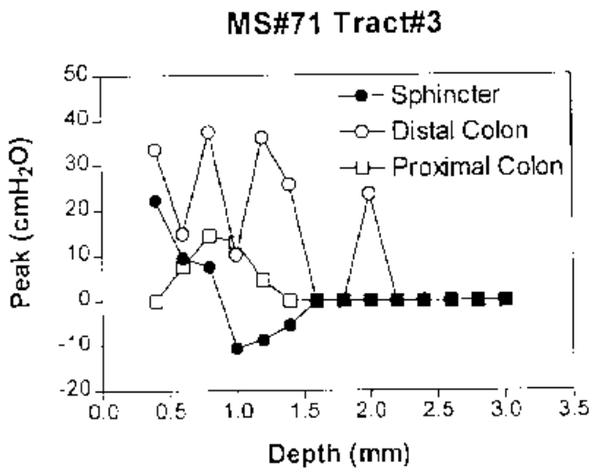

MS#71 Tract#2
Duration (sec)
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

Figure 7


MS#71 Tract#3
Area (cmH2O•sec)
1200
1000
800
600
400
200
0
-200
-400
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon


MS#71 Tract#3
Peak (cmH2O)
50
40
30
20
10
0
-10
-20
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

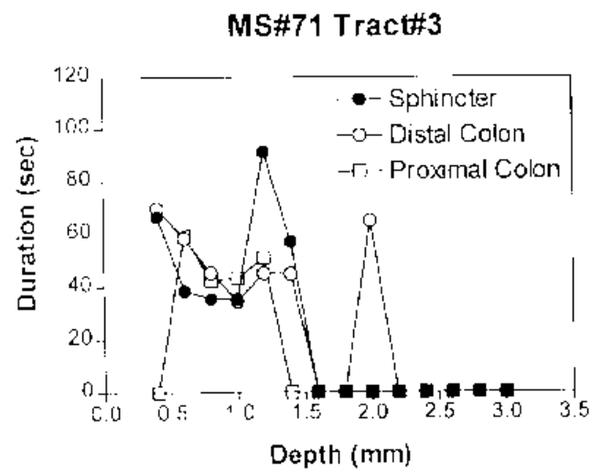
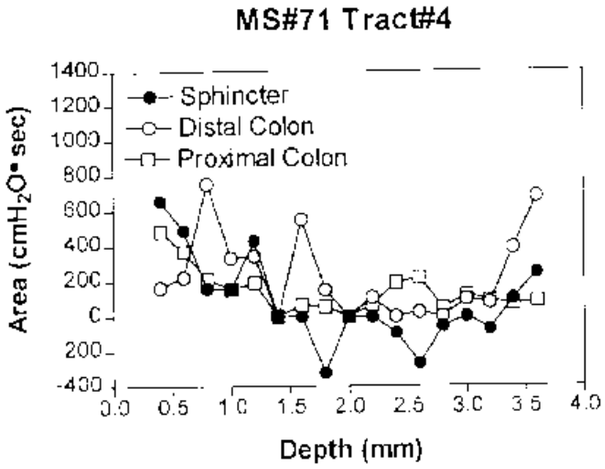
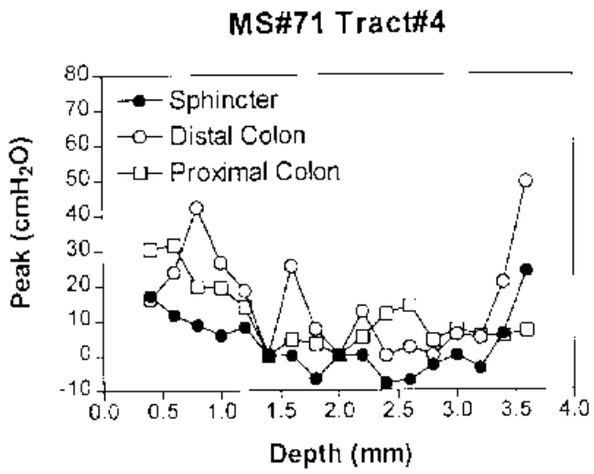

MS#71 Tract#3
Duration (sec)
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Depth (mm)
Sphincter
Distal Colon
Proximal Colon

Figure 8


MS#71 Tract#4
Sphincter
Distal Colon
Proximal Colon
Area (cmH2O*sec)
1400
1200
1000
800
600
400
200
0
-200
-400
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Depth (mm)


MS#71 Tract#4
Sphincter
Distal Colon
Proximal Colon
Peak (cmH2O)
80
70
60
50
40
30
20
10
0
-10
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Depth (mm)

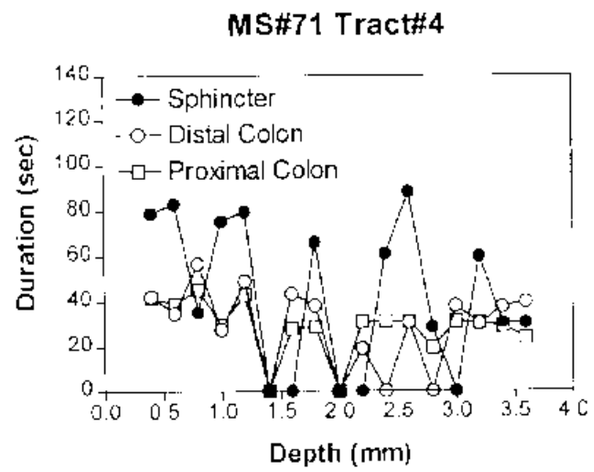
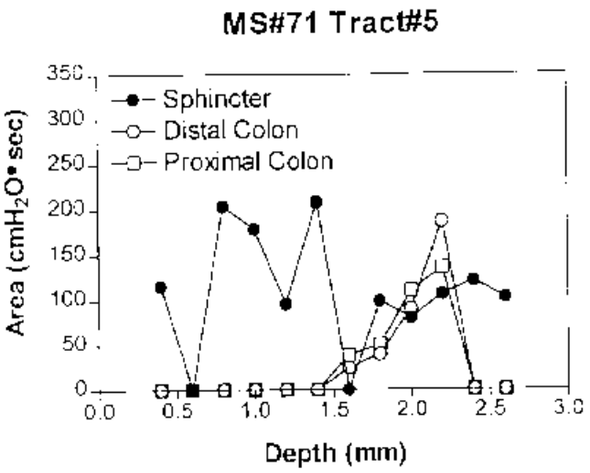
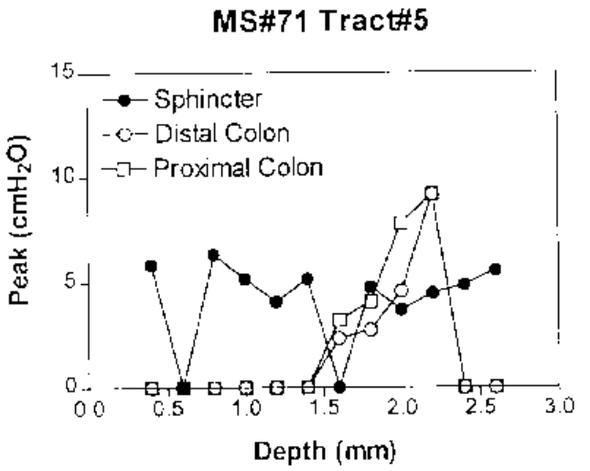

MS#71 Tract#4
Sphincter
Distal Colon
Proximal Colon
Duration (sec)
140
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Depth (mm)

Figure 9


MS#71 Tract#5
Sphincter
Distal Colon
Proximal Colon
Area (cmH2O•sec)
350
300
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Depth (mm)


MS#71 Tract#5
Sphincter
Distal Colon
Proximal Colon
Peak (cmH2O)
15
10
5
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Depth (mm)

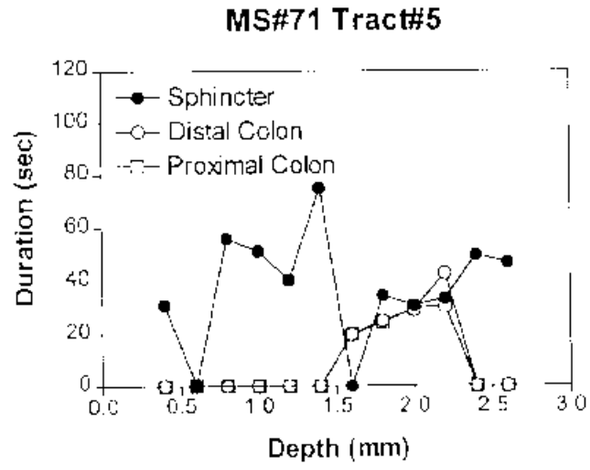

MS#71 Tract#5
Sphincter
Distal Colon
Proximal Colon
Duration (sec)
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Depth (mm)

Figure 10